Susceptibility Testing of Oritavancin against Vancomycin Resistant, Daptomycin non-Susceptible *Enterococcus faecium* Utilizing Commercially Available Gram-Positive Plates

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INTRODUCTION

Vancomycin-resistant *enterococcus faecium* (VRE) is a leading pathogen and a major therapeutic hurdle in immunocompromised patients, particularly those receiving hematopoietic stem cell transplant (HSCT). VRE is the most common gram-positive organism isolated from febrile, neutropenic patients and those with pre-engraftment bacteremia after allogenic HSCT.[1-4] VRE bacteremias in transplant recipients have been associated with mortality rates of 9-22%.[5,6]

Few drugs have been approved for treatment of VRE. Daptomycin has been used "off label" for the treatment or salvage therapy for VRE infections including bacteremias. In addition to lack of comparative efficacy, emergence of non-susceptible isolates has been reported.[7-10] Mechanisms for daptomycin resistance have not been fully explained, although several genetic pathways have been described[8]. In a 2011 report by Kamboj et al., daptomycin-resistant VRE bacteremia at Memorial Sloan Kettering Cancer Center (MSKCC) increased from 3.4% in 2007 to 15.2% in 2009.[7] Among 78 patients with VRE bacteremia at MSKCC between Jan. 1, 2012 - Dec. 31, 2013, 53(68%) had an initial isolate with daptomycin MIC ≥ 4 mcg/mL.

Oritavancin is a semi-synthetic lipoglycopeptide that has been FDA-approved for the treatment of adult patients with acute bacterial skin and skin structure infections caused by susceptible isolates of: *Staphylococcus aureus* (including methicillin-susceptible and methicillin–resistant isolates); *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus anginosus* group , and *Enterococcus faecalis* (vancomycin-susceptible isolates only).[4]

Oritavancin has shown *in vitro* activity against VRE, including *Enterococcus faecium* that have reduced susceptibility to Daptomycin.[11] However, breakpoints for this organism are not defined for Oritavancin.

The study objective is to test *in vitro* susceptibilities of daptomycin non-susceptible vancomycin-resistant *Enterococcus faecium* with a daptomycin MIC of ≥ 4 mcg/mL isolated from bacteremic cancer patients using commercially available Sensititre™ gram positive plate (FDANPF).

MATERIALS AND METHODS

Strain collection: 61 archived bacterial isolates of vancomycin-resistant *Enterococcus faecium* with daptomycin MIC ≥4 mcg/mL were identified. Isolates were collected from oncology patients with sustained bloodstream infections (BSI) (defined as ≥ 48 hours of bacteremia) between 2009-2015 at MSKCC.

Susceptibility testing: Sixty-one unique VRE blood isolates with daptomycin MIC ≥ 4 mcg/mL isolated from patients who developed bacteremia at MSKCC between 2009-2015 were included in the study. Testing was performed in accordance with Sensititre ™ plate (FDANPF; Thermo Fisher) instructions. Colony count was confirmed for each plate. The *Enterococcus Faecalis* ATCC control strain 29212 was run on each day of testing, according to CLSI M07-A10 and CLSI M100-S25 standards for broth microdilution method and interpretation.

MATERIALS AND METHODS (Cont.)

Susceptibility testing was simultaneously performed for oritavancin, range: ≤0.002 to ≥4 mcg/mL; telavancin, range: ≤0.001 to ≥2 mcg/mL; celfaroline, range: ≤0.06 to ≥64 mcg/mL ; vancomycin, range: ≤0.25 to≥128 mcg/mL; dalbavancin, range: ≤0.008 to ≥ 2 mcg/mL; clindamycin, range: ≤0.5 to ≥2 mcg/mL; tedizolid range: ≤0.03 to ≥ 4 mcg/mL; erythromycin range: ≤ 0.5 to ≥4 mcg/mL; linezolid, range: ≤0.25 to ≥32 mcg/mL; and oxacillin range: ≤0.06 to ≥4 mcg/mL.

Figure 1. Sensititre ™ Gram Positive Plate Format

	1	2	3	4	5	6	7	8	9	10	11	12
A	ORI 0.002	ORI 0.004	ORI 0.008	ORI 0.015	ORI 0.03	ORI 0.06	ORI 0.12	ORI 0.25	ORI 0.5	ORI 1	ORI 2	OR 4
B	TLA 0.001	TLA 0.002	TLA 0.004	TLA 0.008	TLA 0.015	TLA 0.03	TLA 0.06	TLA 0.12	TLA 0.25	TLA 0.5	TLA 1	TLA 2
C	CPT 0.06	CPT 0.12	CPT 0.25	CPT 0.5	CPT 1	CPT 2	CPT 4	CPT 8	CPT 16	CPT 32	CPT 64	FOXS 6
D	VAN 0.25	VAN 0.5	VAN 1	VAN 2	VAN 4	VAN 8	VAN 16	VAN 32	VAN 64	VAN 128	DT1	DT2
E	DAL 0.008	DAL 0.015	DAL 0.03	DAL 0.06	DAL 0.12	DAL 0.25	DAL 0.5	DAL 1	DAL 2	CLI 0.5	CLI 1	CLI 2
F	TZD 0.03	TZD 0.06	TZD 0.12	TZD 0.25	TZD 0.5	TZD 1	TZD 2	TZD 4	ERY 0.5	ERY 1	ERY 2	ERY 4
G	LZD 0.25	LZD 0.5	LZD 1	LZD 2	LZD 4	LZD 8	LZD 16	LZD 32				NEG
H	OXA+ 0.06	OXA+ 0.12	OXA+ 0.25	OXA+ 0.5	OXA+ 1	OXA+ 2	OXA+ 4			POS CON	POS CON	POS CON

FOXS	Cefoxitin screen	DAL	Dalbavancin	TZD	Tedizolid
CPT	Ceftaroline	ERY	Erythromycin	TLA	Telavancin w/ tween mimic
CLI	Clindamycin	LZD	Linezolid	VAN	Vancomycin
DT1	D test 1	ORI	Oritavancin	NEG	Negative Control
DT2	D test 2	OXA+	Oxacillin+2&NaCl	POS	Positive Control

RESULTS

Sixty-one isolates of vancomycin-resistant *Enterococcus faecium* with daptomycin MIC ≥ 4 were identified; 22 (36%) of isolates had daptomycin MIC > 4 mcg/mL with the remaining isolates tested had MICs of 4.0 mcg/mL

Each isolate represents unique patient bacteremia episode. Baseline clinical data is listed in Table 2.

Inoculum concentration for each isolate was within CLSI broth microdilution method guidelines

Oritavancin exhibited activity against VRE with median MIC 0.06 mcg/mL (range: 0.08-0.25 mcg/mL) (Figure 1).

MIC-50 for Oritavancin was 0.06 mcg/mL; MIC-90 for Oritavancin was 0.12 mcg/mL.

Median linezolid MIC was 2 mcg/mL (range 1-16). Median tedizolid MIC was 0.5 mcg/mL (range 0.25 to ≥ 4).

Nearly all (60/61, 98%) isolates had ceftaroline MIC ≥64 mcg/mL.

RESULTS (Cont.)

Fifty-seven (93%) of 61 isolates were resistant to clindamycin.

Additionally, 49 (80%) and 59 (95%) of 61 isolates had telavancin and dalbavancin MIC ≥ 2 mcg/mL, respectively.

Figure 1. Oritavancin MIC Distribution

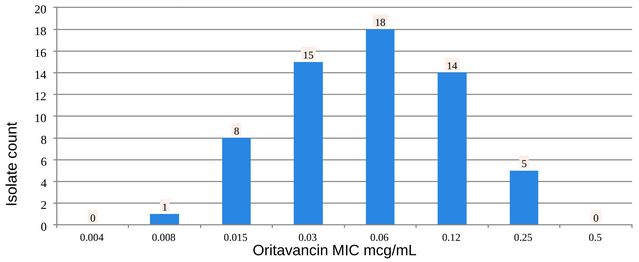

Table 1. Oritavancin MIC (mcg/mL) distributions and frequency (%) against Linezolid MIC (mcg/ml), Tedizolid, Telavancin and Dalbavancin MIC(mcg/mL)

Drug	Isolate count	Oritavancin MIC mcg/mL % isolates			
		≤ 0.03	0.06	0.12	0.25
Linezolid MIC= ≤ 2 mcg/mL	56	43 %	29 %	21 %	7 %
Linezolid MIC= 4 mcg/mL	2	0 %	0 %	50 %	50 %
Linezolid MIC= 16 mcg/mL	3	0 %	67 %	33 %	0 %
Tedizolid MIC= ≤ 0.5 mcg/mL	55	41 %	26 %	24 %	6 %
Tedizolid MIC = 1 mcg/mL	1	0 %	0 %	0 %	100 %
Tedizolid MIC= 2 mcg/mL	3	0 %	67 %	33 %	0 %
Tedizolid MIC = 4 mcg/mL	2	50 %	50 %	0 %	0 %
Telavancin MIC= ≤ 1 mcg/ML	6	83 %	17 %	0 %	0 %
Telavancin MIC= 2 mcg/ML	6	67 %	33 %	0 %	0 %
Telavancin MIC = ≥ 2 mcg/mL	49	31 %	31 %	29 %	9 %
Dalbavancin MIC= 0.12 mcg/mL	1	100 %	0 %	0 %	0 %
Dalbavancin MIC= 0.5 mcg/mL	1	100 %	0 %	0 %	0 %
Dalbavancin MIC= ≥ 2 mcg/mL	57	38%	31 %	24 %	8 %

Table 2. Clinical Data N=61

Male gender (n=34)	56%
Solid Tumor (n=9)	16 %
Hematologic Malignancy (n=52)	84%
Mortality rate at hospital discharge (n=44)	72%

CONCLUSIONS

For VRE isolates with high MIC to daptomycin, the oritavancin median MIC was 0.06 mcg/mL.

Commercially available gram-positive Sensititre™ plates(FDANPF; Thermo Fisher) provide consistent results and can be used to test susceptibility of VRE to Oritavancin.

Oritavancin may have a role in management of serious VRE infections with limited treatment options.

The findings of this investigation support the value of further study on the role of oritavancin in treating vancomycin-resistant *Enterococcus faecium* infections with high daptomycin MIC.

DISCLOSURES

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